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Phenotypic and histopathological tumor characteristics according to *CDKN2A* mutation status among affected members of melanoma families

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Conflict of Interest

The authors state no conflict of interest.

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To the Editor

Highly penetrant mutations in the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene have been identified as major risk factors for melanoma, and they account for between 20% and 50% of familial cases (Kefford *et al.* 1999, Goldstein and Tucker 2001). Pathogenic germline mutations at *CDKN2A* have been associated with malignancies other than melanoma, including: breast and pancreatic cancers (Borg *et al.* 2000, Goldstein *et al.* 2006, de Snoo *et al.* 2008, Ghiorzo *et al.* 2012, Potrony *et al.* 2014), smoking-related cancers of the head and neck, lung cancer, and gastroesophageal carcinomas (Helgadottir *et al.* 2014, Potjer *et al.* 2015), as well as central nervous system tumors (Petronzelli *et al.* 2001, Pasmant *et al.* 2007). Moreover, there is recent evidence to suggest that familial melanoma cases who are wildtype for *CDKN2A* are not at increased risk for non-melanoma cancers in contrast to pathogenic mutation carriers (Helgadottir *et al.* 2014). Distinguishing familial melanoma cases with and without pathogenic *CDKN2A* mutations may serve to heighten awareness of increased risk for other cancers among carriers in melanoma families. Identifying histopathological and other host features that are associated with inherited pathogenic *CDKN2A* mutations may aide in this pursuit and also serve to better characterize melanoma heterogeneity and elucidate important pathobiological differences between carriers and non-carriers of pathogenic *CDKN2A* mutations.

We studied affected members of melanoma families assembled across centers of the GenoMEL consortium and evaluated differences in host and histopathological tumor characteristics between carriers and non-carriers of pathogenic *CDKN2A* mutations. Written informed consent was obtained for each participant, and individual GenoMEL study center investigations were conducted after approval by their respective institutional review boards. To our knowledge, this study is the largest of its kind and incorporates familial melanoma cases from diverse geographical populations.

GenoMEL participants who signed informed consent were asked about their personal melanoma history and to complete a self-administered questionnaire asking about phenotypic characteristics including: hair color, eye color, freckling, nevi, burnability (effect

of acute sun exposure on skin), and tanning ability (effect of chronic sun exposure on skin). A melanoma family was defined by the presence of three or more cases of verified melanoma, or two cases of verified melanoma in first-degree relatives. Histopathological data were abstracted from pathology or other clinical reports; a centralized pathology review was not performed. Germline DNA was screened for mutations in *CDKN2A* (exons 1α, 1β, 2 and 3) as previously described (Harland *et al.* 2008), and pathogenicity was assigned according to Supplemental Table 1. Pathogenicity was based on demonstrated (*i.e.* published) impact on the biological functioning of *CDK2NA*, and putative pathogenicity of specific mutations was based on evidence of cosegregation within melanoma families or bioinformatically inferred impact on *CDKN2A* function. Participants were classified based on presence or absence of a pathogenic or putatively pathogenic variant.

We tested whether differences in levels of histopathological or phenotypic factors exist by *CDKN2A* pathogenic mutation carrier status (α=0.05). Analyses were adjusted for age at diagnosis, gender, study center, and number of affected members per family, and we accounted for the non-independence of observations arising from familial clustering within study center using the repeated subject statement. We also adjusted for presence of any melanocortin-1 receptor (*MC1R)* variant.

There were 1,928 and 1,696 verified cases with *CDKN2A* genotype data who contributed histopathological and phenotypic data to analyses respectively. Associations between *CDKN2A* mutational status and age at diagnosis (P_{trend}<0.0001), multiple primary melanomas (MPM) (P<0.0001), and histologic subtype (P=0.003) were statistically significant after adjustment for covariates and Bonferroni correction (Table 1). Pathogenic mutation carriers were younger at diagnosis and demonstrated higher proportions of MPM and superficial spreading melanomas (SSM) compared to wildtype/nonpathogenic mutation carriers. We also observed statistically significant differences between pathogenic and wildtype/non-pathogenic *CDKN2A* mutation carriers with respect to sun burning (P_{trend}=0.02) and skin type (P=0.04) after adjustment for covariates; pathogenic mutation carriers were significantly less likely to develop severe burns with blistering and more likely to report a darker skin type compared to wildtype/non-pathogenic mutation carriers (Table 2). Neither factor remained significant after Bonferroni correction. Frequencies and p-values for *CDKN2A* association analyses involving all tested histopathological and phenotypic characteristics are reported in Supplemental Tables 2 and 3 respectively.

This study reports an analysis of data collected across all GenoMEL centers using a common protocol. Overall, phenotypic and tumor features were similar among affected family members with and without pathogenic mutations in *CDKN2A*. Nevertheless, these groups were differentiated by some of the same factors that distinguish familial melanomas from those arising in the general population (Florell *et al.* 2005): pathogenic mutation carriers were younger at diagnosis (median age at diagnosis: 38 years vs. 46 years) and they had a greater likelihood of developing multiple melanomas (average number of melanomas: 2.3 vs. 1.4) compared to wildtype/non-pathogenic mutation carriers, findings consistent with results reported by FitzGerald *et al.* (FitzGerald *et al.* 1996). The preponderance of SSM observed among pathogenic mutation carriers is consistent with a recent GenoMEL study by

Sargen *et al.* in which a blinded review of a limited subset of tumors was un (Sargen *et al.* 2015).

It has been suggested that heterogeneity within melanoma is due in part to distinct etiologic pathways—one characterized by increased numbers of nevi, lesion presentation on the trunk, and intermittent sun exposure; and one characterized by fewer nevi, lesion presentation on the head and neck, and chronic sun exposure. Our results provide some evidence for differential effects of acute sun exposure between those with and without pathogenic *CDKN2A* mutations and may suggest that pathogenic mutation carriers are less prone to severe sun burns, a result which is consistent with our observation of a higher proportion of darker skin types reported by pathogenic mutation carriers. However, no differences in nevi or body site of lesion were observed.

Notable limitations of our study were: inability to evaluate the impact of inherited variation at other loci on histopathological and phenotypic factors, ascertainment and sampling of families at some centers was not population-based, centers obtained data to varying degrees, and a lack of centralized pathology review. To address the latter limitation, we conducted a sensitivity analysis restricting histopathological data to those reported by dermatopathologists, who are more likely to report on a fuller spectrum of features relevant to melanoma pathology; the results were not appreciably different from those obtained in our main analysis.

In summary, familial cases with and without pathogenic *CDKN2A* mutations exhibit similar distributions of phenotypic and tumor characteristics. However, cases with pathogenic mutations may be distinguished by features including: younger age at diagnosis, multiple melanoma diagnoses, SSM subtype, and a lack of severe sun burns.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Distribution of host and histopathological tumor characteristics among cases of verified cutaneous melanoma belonging to melanoma families[1] overall and according to *CDKN2A* pathogenicity.

	Overall with *CDKN2A*	**Pathogenic *CDKN2A* mutation carrier**	**Wildtype or non-pathogenic *CDKN2A* mutation carrier**	
	N=1,928	***N=670***	***N=1,258***	**P-value[2]**
	n (%)	**n (%)**	**n (%)**	
Age at Diagnosis				<0.0001
< 30 years	367 (19)	169 (25)	198 (16)	
30–39 years	469 (24)	198 (30)	271 (22)	
40–49 years	384 (20)	138 (21)	246 (20)	
50–59 years	362 (19)	95 (14)	267 (21)	
60–69 years	238 (12)	49 (7)	189 (15)	
≥ 70 years	103 (5)	17 (3)	86 (7)	
missing	5	4	1	
Multiple Primary Melanomas				<0.0001
No	1,297 (67)	346 (52)	951 (76)	
Yes	631 (33)	324 (48)	307 (24)	
missing	0	0	0	
Breslow Depth (mm)†				0.03
in situ	229 (15)	90 (16)	139 (14)	
0.01–1.00	917 (59)	343 (62)	574 (57)	
1.01–2.00	246 (16)	76 (14)	170 (17)	
2.01–4.00	127 (8)	35 (6)	92 (9)	
> 4.00	44 (3)	10 (2)	34 (3)	
missing	365	116	249	
Histologic Subtype				0.003
SSM	879 (71)	378 (73)	501 (70)	
LMM	49 (4)	10 (2)	39 (5)	
NM	104 (8)	31 (6)	73 (10)	
NOS	177 (14)	90 (18)	87 (13)	
Other‡	24 (2)	6 (1)	18 (3)	
missing	695	155	540	

[1] A melanoma family is defined by three or more blood relatives with verified cutaneous melanoma diagnoses or two first degree relatives with verified cutaneous melanoma diagnoses. Verification was made by: pathology report (77%), physician letter or clinical document verifying melanoma diagnosis (20%), cancer registry data (3%), or death certificate (<1%). Individuals who were missing data for all histopathological features were excluded from analysis (n=180).

[2] P-value corresponds to a score test with α=0.05 testing for a difference in proportions between wildtype/non-pathogenic and pathogenic *CDKN2A* mutation carriers with respect to a histopathological feature, with adjustment for age at diagnosis (continuous), sex, number of affected

members per family, study center and familial clustering within study center. All analyses were conducted using SAS v.9.3 (SAS Institute, Cary, NC).

[†]Adjusted for body site of melanoma

[≠]Includes acral lentiginous melanomas and rare subtypes including: nevoid, spitzoid, and desmoplastic melanomas

Table 2

Distribution of phenotypic characteristics among cases of verified cutaneous melanoma belonging to melanoma families[1] overall and according to *CDKN2A* pathogenicity.

	Overall with *CDKN2A*	**Pathogenic *CDKN2A* mutation carrier**	**Wildtype or non-pathogenic *CDKN2A* mutation carrier**	
	N=1,696	***N=604***	***N=1,092***	**P-value[2]**
	n (%)	**n (%)**	**n (%)**	
Effect of Acute Sun Exposure on Skin				0.02
Tan, no burn	47 (4)	18 (5)	29 (3)	
Mild burn	479 (35)	156 (39)	323 (33)	
Burn, then peel	584 (43)	168 (42)	416 (43)	
Severe burn, then blister	263 (19)	56 (14)	207 (21)	
missing	323	206	117	
Skin Type				0.04
Brown/Olive	130 (8)	62 (11)	68 (6)	
Fair	1,144 (71)	384 (68)	760 (72)	
Very Fair	336 (21)	115 (21)	221 (21)	
missing	86	43	43	

[1] A melanoma family is defined by three or more blood relatives with verified cutaneous melanoma diagnoses or two first degree relatives with verified cutaneous melanoma diagnoses. Verification was made by: pathology report (77%), physician letter or clinical document verifying melanoma diagnosis (20%), cancer registry data (3%), or death certificate (<1%). Individuals who were missing data for all phenotypic characteristics were excluded from analysis (n=412).

[2] P-value corresponds to a score test with α=0.05 testing for a difference in proportions between wildtype/non-pathogenic and pathogenic *CDKN2A* mutation carriers with respect to a phenotypic characteristic, adjusted for age at diagnosis (continuous), sex, *MC1R* variant carriage, number of affected members per family, study center and familial clustering within study center. All analyses were conducted using SAS v.9.3 (SAS Institute, Cary, NC).